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SOME PROTOZOA OF ESPECIAL INTEREST FROM VAN CORTLANDT PARK, NEW YORK.

GARY N. CALKINS.

BIOLOGISTS in the vicinity of New York are particularly fortunate in having in Van Cortlandt Park a pond which yields a great variety of interesting forms belonging to the lowest classes of animal life. For the casual passer-by the pond is an unsightly spot, but the green scum which covers parts of its surface, and the healthy water-plants around its edges indicate to the microscopist a rich fauna.

Van Cortlandt Park is about nine miles from the Grand Central Station at Forty-second Street, New York, and is reached by way of the Putnam Division of the New York Central Railroad. The trains on this division leave New York at the terminal of the Elevated Railroad at Eighth Avenue and One Hundred and Fifty-fifth Street at least twice each hour, and from this point Van Cortlandt is only four miles. The pond lies in front of the station at Van Cortlandt, extending away to the northeast. Following the railroad up to the northern end of the pond, beyond the spot where an offshoot of the pond passes below the track to the left, a thick bed of lily pads on the right indicates the spot where *Amœba proteus* can be invariably found. A quantity of the superficial slime covering the bottom should be scooped up in a Mason jar, or other collecting jar which has been used only for living things, and with a small amount of water carried back to the laboratory. Immediate examination shows isolated forms of different genera of Protozoa, which will be seen again later in considerable numbers. Among these will be an occasional Amœba, Actinosphærium, Stentor, etc., — forms commonly found in similar pond water. If the material be emptied into flat dishes about 2″ deep and 12″ to 15″ in diameter, and covered with about an inch and a half of tap water, the majority of the forms originally observed will disappear within a few days, the tap water apparently not agreeing with them. Examination of the material from time to time during the ensuing month, however, shows the gradual return of the original organisms, and with this return the animals are much more numerous than before, and with the advantage that only one type of organism appears to occupy the stage at a time. *Amœba proteus* comes along from four to six weeks after collection, and so constant is this appearance that I can plan in September for my classes in November.

The addition of new tap water has a retarding effect upon the cultures, and a too rapid culture can be checked by this means. A slow culture, on the other hand, should be kept from evaporating and should not be replenished.

In the present contribution I wish to offer a few notes in regard to some forms of particular interest that have appeared during the year, — forms which, for the most part, are rarely observed and but little known. Among these are Nuclearia, Mastigamœba, Multicilia, Lacrymaria, and a species of Actinobolus. It may be of interest, however, to enumerate some of the other forms that are found every year in this water. Of the Rhizopoda there are four species of Amœba (not counting *A. radiosa*, the young form of *A. proteus*); two species of Pelomyxa, three of Difflugia, one of Centropyxis, Arcella, and Euglypha; and of the Heliozoa, Actinophrys, Actinosphærium, Acanthocystis, Pinacocystis, and the dainty stalked Clathrulina are frequently found. Of the Mastigophora there are several species of Euglena, Phacus, Astasia, Trachelomonas, Peranema, Chilomonas, Cryptomonas, Petalomonas; the beautiful colony form Poteriodendron, and the more common colonies Volvox, Pandorina, Eudorina, Gonium, and Synura. The minute choanoflagellates Monosiga and Codosiga, and the dinoflagellates Peridinium and Glenodinium, with their actively vibrating transverse flagella, occur in greater or less abundance. The more common forms of Ciliata in the pond are: Paramœcium, Dileptus, Bursaria, Stentor, Spirostomum, Enchelys, Chilodon, Prorodon, Coleps, Urocentrum, Loxophyllum, Loxodes, Colpoda, Cœnomorpha, Gastrostyla, Urostyla, Euplotes, Kerona, Trichodina (the last two parasites on *Hydra fusca*), Vorticella, Zoöthamnium, Epistylis. Finally, the Suctoria are represented by the two genera Sphærophrya and Podophrya.

Turning now to the less frequently observed forms, there is, in some cases, apart from the fact that they may be found at Van Cortlandt, little to be said in addition to the existing well-known descriptions. A few remarks as to their mode of life and relationships, however, may not be out of place.

Nuclearia delicatula Cienk.

This form, which was first described by Cienkowsky, has occupied rather a varied position in classification. Placed by some writers with the Heliozoa, by others with the Rhizopoda, it belongs to those forms which, in any artificial classification, must be considered as individuals rather than in groups. The peculiarity of Nuclearia lies in the fact that its pseudopodia, while long and needle-like, and in this respect similar to those of the Heliozoa and the Radiolaria, are at the same time very changeable, like those of the Rhizopoda. When the needle-form pseudopodia are drawn in, the organism moves along like an Amœba and changes its place much more rapidly than the majority of the Heliozoa. When not moving, the animal thrusts out its fine ray-like pseudopodia to a distance equal to one and a half times its body diameter. The animal is restless, however, and retains this condition only a short time, moving off soon with an elongate form and by means of its amœboid processes. Unlike the fine ray-like pseudopodia of the common Heliozoa, the appendages of this form have no axial filaments to give them rigidity, and they frequently branch and sub-branch, while the animal is moving (Fig. 1, *B*). In this condition it surrounds and ingests food particles of various kinds, especially plant cells, euglenoids, etc. I had a very good culture of Nuclearia last year, and had studied the little organism rather carefully for several days, devoting many hours to their mode of life, etc., but I had not seen one divide. One day I had a very fine specimen under an immersion lens, when a colleague, who was born under a lucky star, came in. He had not watched the specimen five minutes before he remarked, "This specimen is beginning to divide." He was generous enough, however, to give me several views of the animal during the process, which lasted only less than a minute and was not unlike the cleavage of a sea-urchin egg. After division the daughter-individuals moved apart with the characteristic amœboid motion.

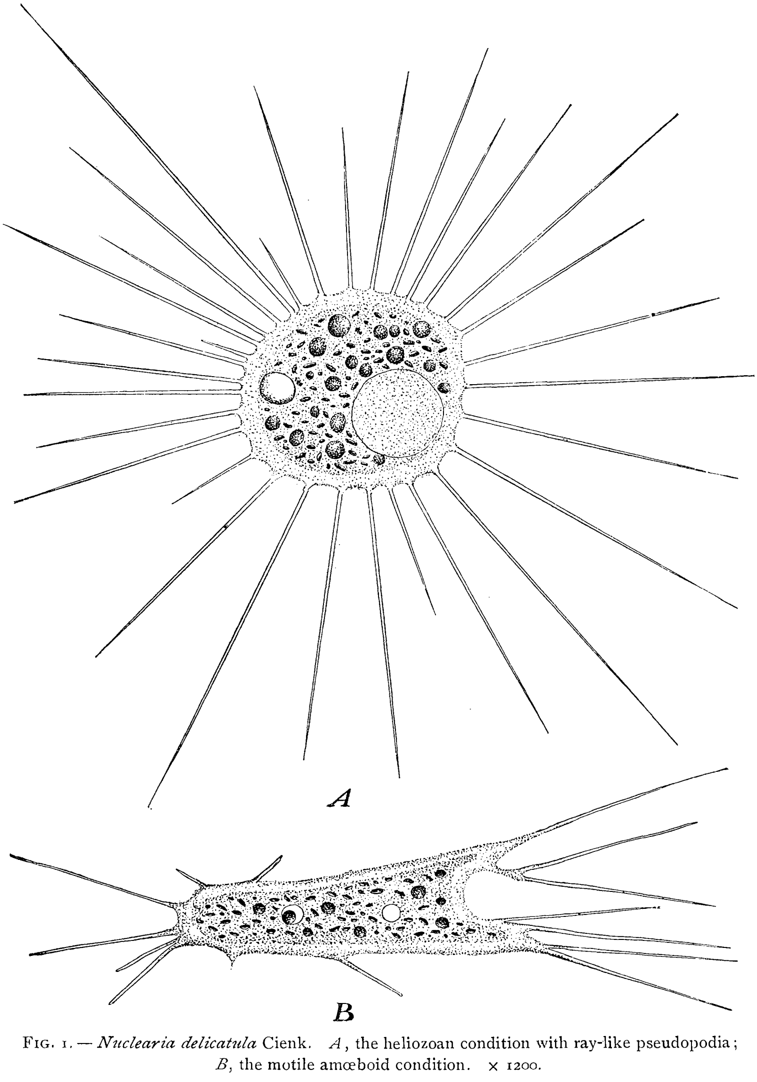

FIG. 1. — *Nuclearia delicatula* Cienk. *A*, the heliozoan condition with ray-like pseudopodia; *B*, the motile amœboid condition. × 1200.

It is not an easy matter to interpret Nuclearia, for it may be regarded as a degenerate heliozoan which has lost its axial filaments, or as a primitive form which has never had these peculiar axial structures. The interpretation involves the old dispute over the most primitive forms of Protozoa. Are we, with Klebs, to hold that the flagellates were the most primitive forms ; or with Lankester, that the rhizopods are to be so considered ; or with Bütschli, that the flagellated amœboid forms were the ancestors of both rhizopods and flagellates? The problem, to my mind, has no great importance, for it is quite probable that the Protozoa have not come down without change from that unknown period of the primitive animal forms, while even in historic times they may have been adapted and readapted many times over to changing conditions of environment. The fact that the majority of Rhizopoda have flagellated swarm-spores is not sufficient proof that their ancestors were flagellated, while on the other hand the frequent amœboid condition of the flagellates is equally valid evidence that the rhizopod form was the older. Flagella and pseudopodia are not far removed from one another, and frequent observations have confirmed Dujardin's early view that flagella may become pseudopodia and pseudopodia flagella. The transitional forms such as Dimorpha, Actinomonas, etc., only strengthen the connection, and it will be shown, I believe, that many of the pseudopodia of Heliozoa are similar to the flagella of the Mastigophora. Blochmann has shown, for example, that in Dimorpha the pseudopodia have axial filaments, which, as in Actinophrys, focus in the nucleus or its vicinity. The flagellum, too, is focused at the same point, so that in Blochmann's figure the axial filaments and the flagellum appear to be the same in structure. Artodiscus, according to Pénard, is a heliozoön which dances about the field upon the tips of its ray-like pseudopodia. In this case the pseudopodia are like so many flagella, for there can be no doubt that the motion is due to the contraction and elasticity of the pseudopodia and probably of the axial filaments in them. Acanthocystis, also, has a slow, rolling motion, by means of which it covers a distance equal to twelve times its own diameter in one minute (Pénard). Here, also, although less than in Artodiscus, the pseudopodia are probably contractile.

There thus appears to be some morphological ground in favor of the hypothesis that Rhizopoda may have originated from the Mastigophora and through the Heliozoa. Artodiscus and Acanthocystis may be regarded as having many flagella of modified form, while Dimorpha, which has a typical flagellate stage, has one true flagellum and at the same time pseudopodia of the heliozoan type. In Actinophrys, Sphærastrum, Heterophrys, etc., the contractile element, whatever it is, is lost, and the pseudopodia appear rigid and apparently serve no purpose in movement. They are of the same type, however, as in Artodiscus and Dimorpha, and, as in the latter, they center at a common point to form a system of rays very much like a centrosphere in Metazoa. In Actinosphærium the rays no longer focus in a common center, although each ends in the vicinity of some nucleus.

In respect of the axial filaments the Foraminifera apparently represent an intermediate stage between the Heliozoa and the fresh-water Rhizopoda. It has been frequently pointed out that the central portion of the pseudopodia of these forms is dense and stiff as compared with the outer plasm, which has the typical flowing motion of a rhizopod, thus reversing the conditions of one of the lobose forms.

If the relations as outlined above would hold good between the Heliozoa and the Mastigophora, then Nuclearia, having lost its axial filaments, finds its real position as an intermediate form between Heliozoa and fresh-water rhizopods, retaining, however, the fine and needle-like pseudopodia of the Heliozoa. Considered in this connection it would be a degenerate form. The entire argument, however, might work equally well the other way, and it might be held that an Amœba such as *A. radiosa*, or *A. tentaculata*, or Nuclearia, develops vibratile pseudopodia having an axial filament of especial contractile substance. The foraminiferan pseudopodia would be a first step in such a development, Acanthocystis, Artodiscus, and Dimorpha successive following steps, until a flagellate would be the outcome. It would be difficult only to place Actinophrys, Actinosphærium, Heterophrys, Sphærastrum, etc., according to such an hypothesis. In either hypothesis Nuclearia would form an important intermediate link.

Mastigamœba verrucosa S. K.

A number of specimens of this interesting form appeared in one of my cultures during October, and I had a good opportunity to study it. The species is different from that described by F. E. Schultze (*Mastigamœba aspera*), and belongs apparently to that which Kent described as *M. verrucosa*, although in size and general form, etc., it agrees with the description of *Mastigamœba invertens* of Klebs. I could not make out, however, that the organism reverses its axes when it comes to rest, — a point upon which Klebs placed considerable importance.

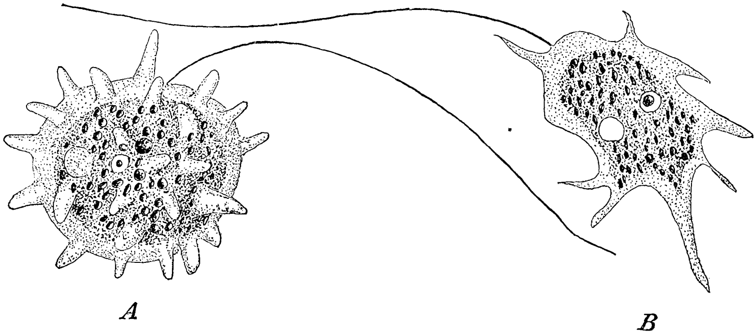

FIG. 2. — *Mastigamœba verrucosa* S. K. *A*, spherical form assumed when swimming with the flagellum in advance; *B*, flattened form with lobose pseudopodia. × 1250.

When first observed this species of Mastigamœba appears like a monad with an irregular outline, which makes its way through the water with a peculiar irregular course, vibrating the while as though shaken vigorously. The cause of the vibration is the action of the rather long flagellum, which is directed in advance. On all sides of the minute body are lobose processes of variable length (Fig. 2). After progressing in this way for some time it finally comes to rest and the pseudopodia are stretched out in all directions, like those of a small Amœba. The flagellum, in the mean time, vibrates slowly at one end. In its usual motile condition the organism is round, but when quiet and with outstretched pseudopodia it assumes a flattened shape, and a distinct ectoplasm and endoplasm can be made out. After a shorter or longer period of rest the action of the flagellum becomes more and more rapid, the body becomes detached from the substratum and assumes a spherical form, after which it again moves through the water with the peculiar vibrating motion which first attracted my attention. A minute contractile vacuole pulsates at irregular intervals and cannot be made out easily when the organism is moving. A small spherical nucleus lies near the base of the flagellum, but no structure could be made out.

In the resting condition the pseudopodia surround and take into the body small objects of different kinds, which are stored up in the endoplasm until digested. The process is apparently no different from that in *Amœba proteus*.

Mastigamœba belongs to a group of flagellated amœboid forms, the Rhizomastigidæ which Bütschli regarded as ancestral to both flagellates and rhizopods. Klebs, on the other hand, placed the beginnings of both groups farther back, and considered the Rhizomastigidæ as flagellates which have become amœboid.

Multicilia

Only one individual of this interesting genus was seen, and no details were made out save the presence of about twenty long flagella-like cilia distributed evenly about the body. By means of these appendages the organism moved along with a rolling motion.

The search for the beginnings of the ciliate stem from some more generalized organisms has been singularly vain. The conservative structure which the infusorian body presents gives but little clue to their ancestry. In only one other known case (Polykrikos among the Dinoflagellidia) is there a similar nuclear dimorphism, while the almost universal reproduction by transverse division is met with among the Mastigophora but rarely. It is generally conceded that the infusorian stem must have arisen from the flagellate stem at an early period, and must have become progressively differentiated until the present striking peculiarities were well established. It is assumed by Bütschli and by the majority of others who adopt the hypothesis that the ciliates were derived from the Mastigophora, that the original forms may have been similar to the present genus Multicilia, whose motile organs are more like flagella than they are like cilia. There is no close connection between the cilia and flagella, as there is between the latter and pseudopodia, but Bütschli holds that the Ciliata might have been derived from such a form as Multicilia, in which the motile organs became progressively shortened and increased in number.

There is another hypothesis which has been maintained by Entz and Maupas amongst others, in which the Ciliata are derived from the Rhizopoda through such forms as Actinobolus and the Enchelinidæ in general, two types of which have been found in Van Cortlandt water, Lacrymaria and Actinobolus. Before speaking of this hypothesis in detail I will add a few notes on these two genera.

Lacrymaria olar Ehr.

Of all known micro-organisms, *Lacrymaria olar* is the most remarkable for its elasticity, and manner in which it stretches out a neck-like portion bearing the mouth. The wonderful variability of this portion of the body cannot be represented in a figure, but when one has witnessed the contortions which it undergoes he will never forget the organism. Sometimes the "neck" is stretched forwards in a straight line to a distance equal to six times the diameter of the body ; again it may be wound around the body of the animal from the insertion point to the posterior end of the animal, and forward again on the other side ; and even then it may stretch out to a considerable distance in advance of the forward end ; or, on the other hand, it may be retracted until the mouth appears to be merely the extremity of an ovoid body.

Lacrymaria is not an uncommon form, and in the present connection it is mentioned chiefly for its bearing upon the hypothesis as to the origin of the Suctoria.

Actinobolus radians St.

This infusorian is much more rare than Lacrymaria, and from its rarity perhaps more interesting. It was first described by Stein, then by Entz, who studied it very carefully, and later by Von Erlanger, who made out a number of minor points of structure. It is almost always found in the company of certain forms of Suctoria, Von Erlanger alone not finding these associates. In the Van Cortlandt water the organism was overlooked for some time, because of its supposed identity with the suctorian Sphærophrya, which was present in considerable numbers. Actinobolus is a small spherical organism not much larger than a Urocentrum, and when at rest resembles a Sphærophrya, which in turn resembles an Actinophrys. It is only when this form is studied with a medium high power that a fringe of vibrating cilia can be made out around the profile of the periphery. It may then be seen that there are groups of long cilia (ten or twelve to a group) arranged about the base of each of the long tentacle-like processes, which give to the organism, when at rest, the aspect of a heliozoön (Fig. 3). The tentacles are the most interesting feature of the ciliate, and their function has been in considerable doubt. Stein was unable to assign any function, but described them as retractile, like the tentacles of the Suctoria, to which he compared them, observing at the same time that they might be entirely withdrawn into the body, leaving no trace of their former presence. Entz thought that they might assist the organism in getting

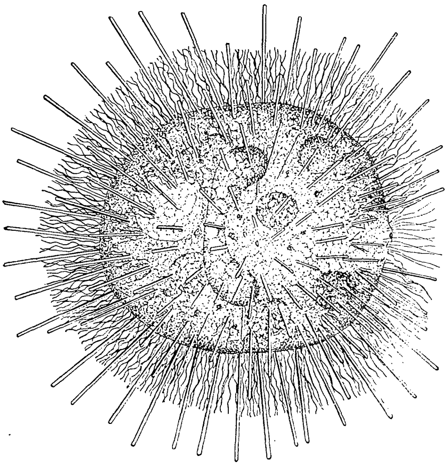

FIG. 3.— *Actinobolus radians* St. The tentacles are partially drawn in and the animal is represented from the side with the small mouth in advance. The nucleus is elongate and constricted. Six Halteria are faintly represented in the endoplasm. × 1200.

food, and observed that the ends of the tentacles were attached to threads of algæ which appeared to be ruptured at the points touched, and he concluded that a secretion of some sort is poured out upon the alga by the tentacle. He also maintained that the organism is exclusively a plant-eater. Von Erlanger described the tentacles as possessing a stiff dart or needle-like trichocyst at the end ; these, he said, are drawn in with the tentacle until, when the tentacles are entirely withdrawn, they form a peripheral layer of trichocysts as in a Prorodon. He also observed that the organism may swallow other living forms and described the seizure of a small flagellate by the cilia about the mouth opening.

There are a few points which I may be able to make in addition to the above. The organism, when feeding, lies attached to the bottom of the vessel by its oral tentacles (*i.e.*, those about the mouth, which are no different from the others on the animal), and mouth downwards. This brings the posterior end of the organism upward, and the anal spot and contractile vacuole can be easily seen and studied while the animal is in this position. The vacuole is not in the center of the dorsal surface, but lies a little to one side (Fig. 4). The tentacles are almost as distensible as the neck of Lacrymaria, and may stretch out a distance of two and a half diameters of the organism, or retract until they disappear in the body. They arise from the substance of the cortical plasm, which is covered by a very thin cuticle or outer membrane, but they cannot be traced into the inner plasm. The cilia are more like flagella than cilia, and move with a vibratory rather than a stroking motion ; while resting they are easy to follow, but when the tentacles are all withdrawn the motion becomes much more rapid, and only the general waving surface can be made out.

It is when the animal is quiet that it takes in food. It remains for hours, sometimes, without giving a sign of feeding, but the tentacles are stretched out to their greatest length and it appears to be merely waiting for something to come. Things do come, especially flagellates like Chilomonas, Trachelomonas, Euglena, etc., and ciliates such as Urocentrum, Paramœcium, Bursaria, Colpoda, etc., but no attention is paid to them. Finally, however, a particular kind of ciliate comes along, *Halteria grandinella*, which, sailing along with its graceful but spasmodic motion, suddenly finds itself on the end of one of the long tentacles of Actinobolus. The captive whirls round and round for a brief second or so, and then becomes quiet, just as it does when captured in a similar way upon the tentacles of a suctorian. The tentacle then begins to shorten, and as it shortens the victim is drawn towards the captor,

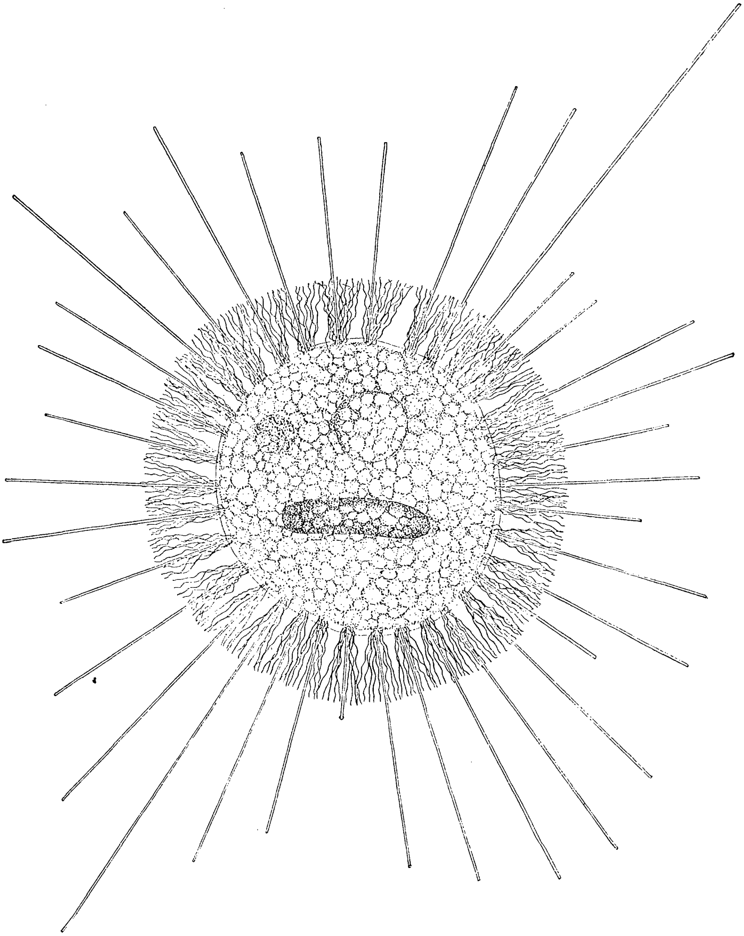

FIG. 4. — *Actinobolus radians* St. at rest, with tentacles widely outstretched, waiting for Halterias, one of which has been captured. The mouth is on the underside. × 1200.

perhaps to the side, perhaps on top ; wherever the prey may be drawn, however, it is gradually worked downwards by the tentacles to the underside toward the mouth, and then with a single swallow it is transferred to the waiting endoplasm in which it is digested. So insatiable is the appetite of this form that, after adding water containing Halteria in abundance, I have seen it swallow in the manner described above no less than ten individuals within half an hour, after which its endoplasm appeared filled with foreign bodies, as Von Erlanger described in the form he observed.

This then is the function of the tentacle, a food-catching organ or set of organs ; the trichocyst which Von Erlanger described, or its analogue (I was unable to make it out as described by Erlanger), brings down the prey, and the tentacle, by shortening, fetches it to the mouth. The tentacle itself is inserted in the Halteria, for it can be seen with the greatest distinctness when the victim becomes quiet, and I believe that the dart at the end as described is not discharged into the prey but is driven into the soft body of the victim as a minute spear, with shaft attached.

When fully fed or when warmed by artificial means the animal detaches itself and draws in its tentacles. It then swims mouth first in a rambling sort of way, turning the while on its longer axis (Fig. 3).

Apart from the general biological interest which such an organism has, there is a considerable interest from a theoretical point of view. Maupas maintained in 1881 that the Ciliata have been derived from the Rhizopoda through the Suctoria, and that the tentacles are to be compared with pseudopodia, while the forms with both cilia and tentacles were considered intermediate. So important was the group of tentacle-bearing forms considered that Mereschowsky formed a special group — the Suctociliata — for their reception. Neither Maupas, Entz, nor Mereschowsky could regard the tentacles in these forms as food-taking organs similar to those of the Suctoria, and could assign them no function other than that of assisting in the capture of aliments. As well known, the tentacles in the Suctoria are for the most part hollow, and the food is taken in some manner through the open ends. Maupas regarded them, together with the tentacles of Actinobolus, Ileonema, and Mesodinium, as pseudopodia, and thought that through them the ciliates came from the Suctoria and the latter from the Rhizopoda. This theory of the origin of the Ciliata from Rhizopoda has but an historical interest, and it is now very generally if not almost universally held that the Suctoria came from the Ciliata ; while the tentacled forms of ciliates are merely independent modifications for special ends and with no phylogenetic significance. A number of minor points suggest the close relation of the forms like Actinobolus to the Suctoria, but more careful consideration leaves little reason to doubt the view that they represent merely parallel evolution, being adaptive features for special ends.